REMARKS

Claims 8-34, 50, 51, 53-92, 118, 119 and 159-162 are pending in the present application. Claims 1-7, 35-49, 52, 93-117, 120-158, and 163-177 were cancelled. By this Amendment, claims 50, 51, 53, 55-57, 60, 68, 69, 72, 74, 75, 82, 84-86, 88, 89, 91, 92, 118, 119, and 159 have been amended. Support for the amendment of claim 86 is found in the specification, *inter alia*, on page 52, lines 23-24. Claims 118 and 159 are amended to be in independent form.

With respect to all claim amendments and cancellations, Applicants have not dedicated or abandoned any unclaimed subject matter and moreover have not acquiesced to any rejections and/or objections made by the Patent Office. Applicants reserve the right to pursue prosecution of any presently excluded claim embodiments in future continuation and/or divisional application.

Attached hereto is a marked-up version of the changes made to the specification by the current amendment. The attached pages are captioned "**VERSION WITH MARKINGS TO SHOW CHANGES MADE**".

Supplemental IDS

Applicants note that a Supplemental Information Disclosure Statement is submitted with this response. The reference (U.S. Pub. No. 20020028497) submitted in the Supplemental IDS is an English counterpart of FR 2 750 433, which was previously submitted to the Examiner on April 5, 2002.

Objections to Claims

Claims 118 and 159 are objected to as being dependent upon a cancelled claim. Claims 118 and 159 have been amended to be independent from the cancelled claims. Applicants respectfully request the objections to claims 118 and 159 be withdrawn.

Rejections under 35 U.S.C. §112, second paragraph

Claims 50, 51, 53-92 as well as the claims that depend from them are rejected under 35 U.S.C. §112, second paragraph, as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter which application regards as the invention.

Specifically, the Examine alleges that the term "rAAV pro-vector" is indefinite because it is not clear whether the terms "rAAV pro-vector" and "rAAV vector" are different or the same. Applicants respectfully note that claims 50, 53, and 55-57 have been amended to replace the term "rAAV pro-vector" with the term "rAAV vector", which renders this rejection moot. Applicants respectfully request the rejection be withdrawn.

The Examiner also alleges that the term "split-packaging gene" in claim 60 is indefinite because the meaning of the term is not clear. Applicants respectfully submit that the meaning of the term "split-packaging gene" is clear for one skilled in the art. In the field of AAV research, the term "split-packaging gene" refers to a recombinant gene encoding one or more AAV packaging proteins (including AAV Rep proteins and/or AAV Cap proteins) wherein the split-packaging gene has been separated from one or more AAV packaging genes to which it is normally linked in the AAV genome. Applicants respectfully request the rejection be withdrawn.

The Examiner alleges that the term "SP resin" in claim 82 is indefinite because it is not defined in the claim or specification. Applicants respectfully submit that the meaning of the term "SP resin" is clear for one skilled in the art. Claim 82 has been amended to recite "a sulfopropyl (SP) resin". Applicants respectfully request the rejection be withdrawn.

Rejections under 35 U.S.C. §103(a)

A. Claims 8-34, 118, 119, and 159-162 are rejected under 35 U.S.C. §103(a) as allegedly being unpatentable over Tamayose et al., further in view of Fanget et al., and O'Riordan et al. The Examiner states that Tamayose et al. teach that rAAV can be purified from lysed cells and supernatant using sulfonated cellulose chromatography and teach that technique

alone is not sufficient for purification. Office Action, page 3. The Examiner states that Fanget et al. teach use of cation and anion exchange chromatography columns in combination to purify virus for vaccine use. Office Action, page 3. The Examiner states that O'Riordan et al. teach use of filtration to clarify lysates and ion-exchange and other types of columns to purify and concentrate rAAV particles, and use of heparin and cation columns to purify adenovirus. Office Action, page 4. The Examiner contends that one of skill in the art, knowing the rAAV of Tamayose required more purification, would look for additional methods to add to the steps in the purification procedure and both O'Riordan and Fanget provide additional mediums to purify the rAAV (anion and cation resins include N-charged amino, TMAE resin, sulfo-resin, heparin sulfate, and DEAE resin). Office Action, page 4. The Examiner further contends that it would have been routine experimentation to use a variety of different chromatographic materials to determine which ones and which combinations give the highest purity, highest percent recovery, and highest titer of infectious rAAV. Office Action, page 4. The Examiner states that it would have been *prima facie* obvious to one of ordinary skill in the art to obtain high titer, pure stocks of rAAV with a reasonable expectation of success.

Applicants respectfully traverse this rejection.

Applicants respectfully submit that the Examiner has failed to establish a *prima facie* case of obviousness. To establish a *prima facie* case of obviousness, three criteria must be met. First, there must be some suggestion, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Third, the prior art reference (or references when combined) must teach or suggest all the claim limitations. These requirements are summarized in the MPEP (MPEP §2143, and §2143.01 to §2143.03), and are based on well-settled case law: *In re Fine*, 837 F.2d 1071, 5 USPQ2d 1596 (Fed. Cir. 1988); *In re Jones*, 958 F.2d 347, 21 USPQ2d 1941 (Fed. Cir. 1992); *In re Merck & Co., Inc.*, 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986); and *In re Royka*, 490 F.2d 981, 180 USPQ 580 (CCPA 1974).

Applicants respectfully submit that the cited references do not provide the motivation to combine reference teachings. As stated above, in order to render a claimed invention obvious there must be some teaching or suggestion, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art supporting the combination of references. Claims 8-34 recite a method of isolating rAAV particles using a combination of a positively-charged anion exchange chromatography and a negatively-charged cation exchange chromatography. Claims 118, 119, and 159-162 recite a step of purifying rAAV particles using a positively-charged anion exchange resin followed by either a cation exchange resin or by tangential flow filtration.

Tamayose et al. do not teach or suggest purification of rAAV using a combination of opposing ion exchange chromatography or a combination of anion exchange chromatography and tangential flow filtration. Fanget et al. only generally teach a method for purifying viruses from a cell line culture by a combination of an anion exchange chromatography step and a cation exchange chromatography step. The type of purification taught by Fanget et al. is directed to purifying virus from proteins and DNA originating from the culture cells used for virus production. Fanget et al. do not teach or suggest that their two-step ion-exchange chromatography purification method is applicable to or useful for rAAV purification. Fanget et al. (U.S. Pat. No. 6,008,036 which is equivalent to PCT WO97/06243 cited by the Examiner), column 1, lines 35-41. Fanget et al. do not teach or suggest that their two-step ion-exchange chromatography can be used for separation of rAAV from helper virus, such as adenovirus, and helper virus proteins. They do not address the purification problem of separation from helper virus or its proteins, which is particularly applicable to rAAV purification. In addition, Fanget et al. do not teach or suggest purification of rAAV using a combination of anion exchange chromatography and tangential flow filtration. O'Riordan et al. teach use of ion-exchange chromatography to purify AAV. The Examiner states that O'Riordan et al. disclose the use of heparin and cation columns to purify adenovirus, which is not AAV. Office Action, page 4. However, O'Riordan et al. do not teach or suggest purification of rAAV using a combination of

opposing ion exchange chromatography or a combination of anion exchange chromatography and tangential flow filtration. Thus, one of ordinary skill in the art would not be motivated to combine the teachings of Tamayose et al. with Fanget et al., and O'Riordan et al. On this ground, the obviousness rejection may be properly withdrawn.

To establish a *prima facie* case of obviousness, there must be a reasonable expectation of success. Tamayose et al. and O'Riordan et al. provide no suggestion of using a combination of opposing ion exchange chromatography or a combination of anion exchange chromatography with tangential flow filtration to purify rAAV. Fanget et al. teach that use of a combination of an anion exchange chromatography step and a cation exchange chromatography step to purify virus from the cellular proteins and DNA from cells used for virus production. Fanget, et al. (U.S. Pat. No. 6,008,036), column 1, lines 35-41; column 2, lines 3-4, and lines 43-45. However, Fanget et al. do not suggest that their two-step ion exchange chromatography is useful in purifying viruses the production of which requires helper virus or helper virus function(s), such as rAAV. Fanget et al. do not teach or suggest that their two-step ion exchange chromatography is useful in purifying rAAV from helper virus or helper virus proteins. In addition, viruses are different in coating proteins, which may have different electric properties. A person skilled in the art would not reasonably expect a purification method that is useful for viruses in general is equally effective in purification of a particular type of virus in view of the potential difference in electric properties of coating proteins, as an example. Based on the above, it would not have been obvious for one of ordinary skill in the art to combine the teachings of those references with a reasonable expectation of success in purifying rAAV particles from not only cellular proteins and DNA but also helper virus and helper virus proteins using a combination of opposing ion exchange chromatography or a combination of anion exchange chromatography with tangential flow filtration. On this ground, the obviousness rejection may be properly withdrawn.

In view of the above, the Examiner has not set forth a *prima facie* case for obviousness for claims 8-34, 118, 119, and 159-162. Applicants respectfully request the rejection of claims 8-34, 118, 119, and 159-162 be withdrawn.

B. Claims 50, 51, 53-92 are rejected under 35 U.S.C. §103(a) as allegedly being unpatentable over Tamayose et al., Fanget et al., and O'Riordan, as applied to claims 8-34, 118, 119, and 159-162 above, and further in view of Shenk. The Examiner states that Shenk et al. disclose a method of producing a rAAV by cotransfecting producer cells having a heterologous DNA flanked by at least one inverted terminal repeat (ITR), helper AAV DNA coding one or more AAV packaging proteins needed for replication and encapsidation, and helper virus, in this case adenovirus. Office Action, page 5. The Examiner acknowledges that Shenk et al. do not teach all the specifics about culture conditions or purification. Office Action, page 5. The Examiner states that Tamayose, Fanget, and O'Riordan teach methods of purification. Office Action, page 6. The Examiner contends that it would have been *prima facie* obvious to grow rAAV under conditions for optimal yield and purify to achieve high titer, pure stocks. Office Action, page 6.

Applicants respectfully traverse this rejection.

Claims 50, 51, 53-92 recite a step of purifying rAAV particles using a positively-charged anion exchange chromatography followed by a cation exchange chromatography or a tangential flow filtration. As discussed in the above section, Tamayose et al., Fanget et al., and O'Riordan et al. do not provide any teaching or suggestion of using a positively-charged anion exchange chromatography followed by a cation exchange chromatography or a tangential flow filtration to purify rAAV particles. The above discussion applies equally here.

The additional reference cited does not cure this deficiency. The Examiner acknowledges that Shenk et al. do not teach all the specifics about culture conditions or purification. Thus, one of ordinary skill in the art would not be motivated to combine the teachings of Tamayose et al., Fanget et al, and O'Riordan et al. with Shenk et al.

To establish a *prima facie* case of obviousness, there must be a reasonable expectation of success. As discussed above, since none of the references cited by the Examiner provides any suggestion of using a positively-charged anion exchange chromatography followed by a cation

exchange chromatography or a tangential flow filtration to purify rAAV particles, it would not have been obvious for one of ordinary skill in the art to combine the teachings of those references with a reasonable expectation of success.

In view of the above, the Examiner has not set forth a *prima facie* case for obviousness for claims 50, 51, 53-92. Applicants respectfully request the rejection of claims 50, 51, 53-92 be withdrawn.

C. Claims 62-64 are rejected under 35 U.S.C. §103(a) as allegedly being unpatentable over Tamayose, Fanget, O'Riordan and Shenk as applied to claims 50, 51, 53-92 above, and further in view of Myers et al. The Examiner states that Myers et al. teach that Ad5ts149 is an efficient helper of AAV in contrast to other temperature-sensitive (ts) adnoviruses examined and the ts viruses at non-permissive temperature grow to 5 logs less titer. Office Action, page 6. The Examiner contends that it would be obvious to one skilled in the art to increase the purity of the rAAV of Shenk by reducing the amount of contaminating helper-virus and to use Ad5ts149 virus to produce rAAV that is more pure. Office Action, pages 6-7.

Applicants respectfully traverse this rejection.

Claim 50, from which claims 62-64 depend, recites a step of purifying rAAV particles using a positively-charged anion exchange chromatography followed by a cation exchange chromatography or a tangential flow filtration. As discussed above, Tamayose et al., Fanget et al., O'Riordan et al., and Shenk et al. do not provide any motivation to combine reference teachings. The above discussion applies equally here.

The additional reference cited does not cure this deficiency. Myers et al. do not provide any teachings or suggestions of purifying rAAV using a positively-charged anion exchange chromatography followed by a cation exchange chromatography or a tangential flow filtration. Thus, Myers et al. do not provide any additional motivation to combine the reference teachings.

In addition, as discussed above since none of Tamayose et al., Fanget et al., O'Riordan et al., and Shenk et al. provide any suggestion of using a positively-charged anion exchange

chromatography followed by a cation exchange chromatography or a tangential flow filtration to purify rAAV particles, and Myers et al. do not provide any teaching or suggestion in this respect, it would not have been obvious for one of ordinary skill in the art to combine the teachings of those references with a reasonable expectation of success.

In view of the above, the Examiner has not set forth a *prima facie* case for obviousness for claims 62-64. Applicants respectfully request the rejection of claims 62-64 be withdrawn.

D. Claim 34 stands rejected under 35 U.S.C. §103(a) as allegedly being unpatentable over Tamayose et al., Fanget et al., and O'Riordan as applied to claims 8-34, 118, 119, and 159-162 above, and further in view of Graham (*J. Gen. Virol.* 1987, 68:937-940). The Examiner states that Tamayose, Fanget, and O'Riordan teach methods of purification of virus and do not teach using suspension cultures for producing rAAV particles. Office Action, page 7. The Examiner states Graham teaches adapting cells to grow in suspension and that cells grown in suspension offer advantages over cells grown in monolayers in terms of efficiency, economy and potential automation of large scale production. Office Action, page 7. The Examiner contends that it would have been *prima facie* obvious for one skilled in the art to use suspension cultures to produce large quantities of rAAV using the method of Graham. Office Action, page 7.

Applicants respectfully traverse this rejection.

Claim 22, which claim 34 depends from, recites a method of isolating rAAV particles using a combination of a positively-charged anion exchange resin and a negatively-charged cation exchange resin. As discussed above, there is no motivation to combine the teachings of Tamayose et al., Fanget et al., and O'Riordan et al. The above discussion applies equally here.

The additional reference cited does not cure this deficiency. Graham does not teach or suggest a method of isolating rAAV particles using a combination of a positively-charged anion exchange resin and a negatively-charged cation exchange resin. Thus, Graham does not provide any additional motivation to combine the reference teachings.

In addition, as discussed above since none of Tamayose et al., Fanget et al., and O'Riordan et al. provide any suggestion of using a combination of a positively-charged anion exchange resin and a negatively-charged cation exchange resin, and Graham does not provide any teaching or suggestion in this respect, it would not have been obvious for one of ordinary skill in the art to combine the teachings of those references with a reasonable expectation of success.

In view of the above, the Examiner has not set forth a *prima facie* case for obviousness for claim 34. Applicants respectfully request the rejection of claim 34 be withdrawn.

E. Claims 87, 88 and 92 are rejected under 35 U.S.C. §103(a) as allegedly being unpatentable over Tamayose, Fanget, O'Riordan and Shenk as applied to claims 50, 51, 53-92 above, and further in view of Graham. The Examiner states that Tamayose et al., Fanget et al., and O'Riordan et al. teach methods of purification of virus and Shenk et al. teach a method to make rAAV, and Shenk et al., Tamayose et al., Fanget et al., and O'Riordan et al. do not teach using suspension cultures for producing rAAV particles. Office Action, page 8. The Examiner states that Graham teaches that cells grown in suspension offer advantages over cells grown in monolayers in terms of efficiency, economy and potential automation of large scale production. Office Action, page 8. The Examiner contends that it would have been obvious for one skilled in the art to use suspension cultures to produce large quantities of rAAV of Shenk et al. using the culture method of Graham. Office Action, page 8.

Applicants respectfully traverse this rejection.

Claim 50, from which claims 87, 88, and 92 depend, recites a step of purifying rAAV particles using a positively-charged anion exchange chromatography followed by a cation exchange chromatography or a tangential flow filtration. As discussed above, Tamayose et al., Fanget et al., O'Riordan et al., and Shenk et al. do not provide any motivation to combine reference teachings. The above discussion applies equally here.

The additional reference cited does not cure this deficiency. Graham does not provide any teachings or suggestions of purifying rAAV using a positively-charged anion exchange chromatography followed by cation exchange chromatography or tangential flow filtration. Thus, Graham does not provide any addition motivation to combine the reference teachings.

In addition, as discussed above since none of Tamayose et al., Fanget et al., O'Riordan et al., and Shenk et al. provide any suggestion of using a positively-charged anion exchange chromatography followed by a cation exchange chromatography or a tangential flow filtration to purify rAAV particles, and Graham does not provide any teaching or suggestion in this respect, it would not have been obvious for one of ordinary skill in the art to combine the teachings of those references with a reasonable expectation of success.

In view of the above, the Examiner has not set forth a *prima facie* case for obviousness for claims 87, 88, and 92. Applicants respectfully request the rejection of claims 87, 88, and 92 be withdrawn.

CONCLUSION

Applicants have, by way of the amendments and remarks presented herein, made a sincere effort to overcome rejections and address all issues that were raised in the outstanding Office Action. Accordingly, reconsideration and allowance of the pending claims are respectfully requested. If it is determined that a telephone conversation would expedite the prosecution of this application, the Examiner is invited to telephone the undersigned at the number given below.

In the unlikely event that the transmittal letter is separated from this document and the Patent Office determines that an extension and/or other relief is required, applicant petitions for any required relief including extensions of time and authorizes the Assistant Commissioner to charge the cost of such petitions and/or other fees due in connection with the filing of this document to **Deposit Account No. 03-1952** referencing docket no. 226272003310.

Respectfully submitted,

Dated: October 11, 2002 By: _____

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"VERSION WITH MARKINGS TO SHOW CHANGES MADE"

In the claims

Please amend claims 50, 51, 53, 55-57, 60, 68, 69, 72, 74, 75, 82, 84-86, 88, 89, 91, 92, 118, 119, and 159 as follows:

50. (Amended) A method of generating a population of recombinant adeno-associated virus (rAAV) particles, comprising the steps of:

[a) providing an AAV producer cell that comprises:

(i) one or more AAV packaging genes, wherein each said AAV packaging gene encodes an AAV replication or encapsidation protein;

(ii) a recombinant AAV (rAAV) [pro-vector] vector that comprises a heterologous non-AAV polynucleotide flanked by at least one AAV inverted terminal repeat (ITR); and

(iii) a helper virus for AAV;]

[b)] a) incubating [the] an AAV producer cell [provided in step a)] under conditions that are permissive for replication of AAV; said producer cell comprising: (i) one or more AAV packaging genes, wherein each said AAV packaging gene encodes an AAV replication or encapsidation protein; (ii) a recombinant AAV (rAAV) vector that comprises a heterologous non-AAV polynucleotide flanked by at least one AAV inverted terminal repeat (ITR); and (iii) a helper virus for AAV;

[c)] b) lysing the producer cell after the incubation of step [b)] a) to produce an AAV producer cell lysate;

[d)] c) chromatographing the AAV producer cell lysate of step [c)] b) on at least one positively-charged anion exchange resin; and

[e)] d) purifying the chromatographic fractions containing rAAV particles of step [d)] c) by cation exchange chromatography or tangential flow filtration to generate a purified population of rAAV vector particles.

51. (Amended) A method of generating a population of rAAV particles according to claim 50, wherein said purifying step [e)] d) comprises subjecting the fractions to cation exchange chromatography.

53. (Amended) A method of generating a population of rAAV particles according to claim 50, wherein said rAAV [pro-vector] vector comprises a heterologous non-AAV polynucleotide flanked by two AAV inverted terminal repeats (ITRs).

55. (Amended) A method of generating a population of rAAV particles according to claim 50, wherein [said providing of the producer cell in step a) comprises introducing] the helper virus is introduced into the producer cell already introduced with the AAV packaging gene(s) and the rAAV [pro-vector] vector.

56. (Amended) A method of generating a population of rAAV particles according to claim 50, wherein [the providing of the producer cell in step a) comprises introducing] the rAAV [pro-vector] vector and the helper virus are introduced simultaneously or sequentially into the producer cell already introduced with the AAV packaging gene(s).

57. (Amended) A method of generating a population of rAAV particles according to claim 50, wherein [the providing of the producer cell in step a) comprises introducing] the AAV packaging gene(s) and the rAAV [pro-vector] vector are introduced simultaneously or sequentially into the [host] producer cell already introduced with the helper virus.

60. (Amended) A method of generating a population of rAAV particles according to claim 50, wherein [the providing of the producer cell in step a) comprises introducing into the producer cell] at least one AAV split-packaging gene is introduced into the producer cell.

68. (Amended) A method of generating a population of rAAV particles according to claim 50, wherein the AAV producer cells of step [b)] a) are concentrated prior to lysis.

69. (Amended) A method of generating a population of rAAV particles according to claim 68, wherein the AAV producer cells of step [b)] a) are concentrated by centrifugation or by tangential flow filtration prior to lysis.

72. (Amended) A method of generating a population of rAAV particles according to claim 50, wherein the AAV producer cell lysate of step [c)] b) is treated with a nuclease prior to chromatography.

74. (Amended) A method of generating a population of rAAV particles according to claim 50, wherein the AAV producer cell lysate of step [c)] b) is clarified prior to chromatography.

75. (Amended) A method of generating a population of rAAV particles according to claim 74, wherein the AAV producer cell lysate of step [c)] b) is clarified by filtration or centrifugation prior to chromatography.

82. (Amended) A method of generating a population of rAAV particles according to claim 51, wherein said cation exchange resin is selected from the group consisting of [an] a heparin sulfate (HS) resin, [an] a sulfopropyl (SP) resin, and a carboxymethyl (CM) resin.

84. (Amended) A method of generating a population of rAAV particles according to claim 50, wherein said step [b)] a) of incubating the producer cell [provided in step a)] is conducted in a vessel selected from the group consisting of a tissue culture flask, a roller bottle, a spinner flask, a tank reactor, a fermentor, and a bioreactor.

85. (Amended) A method of generating a population of rAAV particles according to claim 50, wherein said step [b)] a) of incubating the producer cell [provided in step a)] is conducted using a microcarrier.

86. (Amended) A method of generating a population of rAAV particles according to claim 84 [50], wherein said [vessel] bioreactor is a hollow-fiber, packed-bed or fluidized-bed bioreactor.

88. (Amended) A method of generating a population of rAAV particles according to claim 50, wherein said step [b)] a) of incubating the producer cell [provided in step a)] is conducted in a vessel selected from the group consisting of a spinner flask, a tank reactor and an air lift fermentor.

89. (Amended) A method of generating a population of rAAV particles according to claim 50, wherein said step [b)] a) of incubating the producer cell [provided in step a)] is performed in rAAV medium essentially as shown in Table 2.

91. (Amended) A method of generating a population of rAAV particles according to claim 50, wherein step [b)] a) is conducted for at least 5 days.

92. (Amended) A method of generating a population of rAAV particles according to claim 50, wherein step [b)] a) of incubating the producer cell is conducted in a multi-liter bioreactor and wherein at least about 10^9 replicative units of rAAV per liter of bioreactor volume are isolated after step [e)] d).

118. (Amended) A method of generating a population of [rAAV particles according to claim 117,] recombinant adeno-associated virus (rAAV) particles, comprising the steps of:

a) incubating an AAV producer cell under conditions that are permissive for replication of AAV and which comprise inducing a sub-lethal stress in the AAV producer cell; wherein said AAV producer cell comprising (i) one or more AAV packaging genes, wherein each said AAV packaging gene encodes an AAV replication or encapsidation protein; (ii) a recombinant AAV (rAAV) vector

that comprises a heterologous non-AAV polynucleotide flanked by at least one AAV inverted terminal repeat (ITR); and (iii) a helper virus for AAV;

b) lysing the producer cell after the incubation of step a) to produce an AAV producer cell lysate; and

c) purifying the AAV producer cell lysate to generate a population of recombinant adeno-associated virus (rAAV) particles, wherein said purifying step [d)] comprises chromatographing the AAV producer cell lysate of step [c)] b) on at least one positively-charged anion exchange resin followed by purifying on either a cation exchange resin or by tangential flow filtration to generate a purified population of rAAV vector particles.

119. (Amended) The method of claim 118, wherein said purifying step [d)] c) comprises chromatographing the AAV producer cell lysate of step [c)] b) on at least one negatively -charged cation exchange resin followed by purifying on an anion exchange resin.

159. (Amended) [The method of claim 158] A method of generating a population of recombinant adeno-associated virus (rAAV) particles, [further] comprising the steps of:

a) incubating a producer cell in a cell culture medium under conditions comprising a condition that promotes release of rAAV particles, whereby rAAV particles are released from the producer cell into the culture medium, wherein the producer cell comprises:

(i) one or more AAV packaging genes, wherein each said AAV packaging gene encodes an AAV replication or encapsidation protein;

(ii) a recombinant AAV (rAAV) vector that comprises a heterologous non-AAV polynucleotide flanked by at least one AAV inverted terminal repeat (ITR); and

(iii) helper virus function for AAV;

(b) harvesting the rAAV particles from the cell culture medium, thereby obtaining a population of rAAV particles;

c) chromatographing the rAAV producer cell culture medium on a positively-charged anion exchange resin; and

d) purifying the chromatographic fractions containing rAAV particles of step c) by cation exchange chromatography or tangential flow filtration to generate a purified population of rAAV vector particles.